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<p>(54) Title: HUMAN MAFA</p> <p>(57) Abstract</p> <p>This invention relates to polypeptides, nucleotide sequences, antibodies or fragments thereof, ligands and compositions and their use in the medical fields of inflammation and allergy, disease examples of which include rheumatoid arthritis and asthma. In addition the invention relates to a method for production of the polypeptides. Methods of disease treatment are suggested relying on agents developed in combination with the cloning of the human MAFA molecule. A use of the invention addresses the prevention of cell activation events <i>in vivo</i> which could lead to therapies for the prevention of tumour growth.</p>		

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HUMAN MAFA

Field of Invention

This invention relates to polypeptides, nucleotide sequences, antibodies or fragments thereof, ligands and compositions and their use in the medical fields of inflammation and allergy, disease examples of which include rheumatoid arthritis and asthma. In addition the invention relates to a method for production of the polypeptides. Methods of disease treatment are suggested relying on agents developed in combination with the cloning of the human MAFA molecule. A use of the invention addresses the prevention of cell activation events *in vivo* which could lead to therapies for the prevention of tumour growth.

Nucleotides and amino acid residues are represented herein by their standard codes as identified by the IUPAC-IUB Biochemical Nomenclature Commission and they include all D or L amino acids or analogues and derivatives thereof. The symbol X represents an unidentified amino acid or analogue thereof.

Background to Invention

Mast cells comprise a heterogeneous family of cell types derived from the bone marrow, which are mainly found resident in the connective tissue of the skin, lung and gut. Their common feature is prominent cytoplasmic granules containing heparin, histamine and proteases, which can be released in a process known as degranulation, into the tissues when the cells are appropriately activated. Mast cells are gaining recognition as participants in many inflammatory responses in addition to their documented role in anaphylaxis. However, the biochemical pathways underlying the ability of extracellular stimuli to activate intracellular events still require resolution. After immunological activation via the high-affinity Fc receptors (FcεRI) for immunoglobulin E (IgE) on the surface of the cell, signal transduction

pathways are initiated including the tyrosine phosphorylation of cellular proteins, phosphoinositide hydrolysis, an increase in intracellular calcium, and protein kinase C activation. The mast cell then releases a variety of mediators such as cytokines, lipid-derived mediators, amines, proteases and proteoglycans. These early activation events are believed to be involved in the release of the mediators. The FcεRI receptor is not only expressed on mast cells but also on basophils, langerhans cells, monocytes, and eosinophils, although it is now recognised that the receptor expressed on langerhan cells and monocytes is missing the β chain.

MAFA

An abundant cell surface protein was identified on the surface of the rat basophil leukaemic cell-line RBL-2H3 as a result of monoclonal antibody screening. The antibody used, G63, was later shown to also bind to the surface of mucosal and connective-tissue mast cells (Ortega *et al*, 1991). The cell surface protein was termed mast cell function-associated antigen (MAFA). The cDNA sequence encoding rat MAFA (rMAFA) was isolated by expression cloning (Guthman *et al*, 1995). Rat MAFA is a type II integral membrane glycoprotein that has extensive amino acid homology to calcium dependent (C-type) animal lectins. Interestingly, C-type lectins have been associated with other immunological cell types, CD72 in B lymphocytes, FcεRII (CD23), CD69 in T and B lymphocytes, and Ly-49 and NKR-P1 on natural killer cells.

Recently, the gene structure of rat MAFA has been published along with the sequences of two alternatively-spliced mRNA transcripts (Bocek *et al*, 1997). The full length rat MAFA mRNA is made up from five exons and one of the alternative transcripts lacks the transmembrane exon, exon 2, but maintains the correct reading frame. The other alternatively-spliced transcript lacks both exons 2 and 3 and does not maintain the full length rat MAFA reading frame. No function has yet been assigned to the alternately spliced rat MAFA variants.

Cross-linking of rMAFA on RBL-2H3 cells using G63 monoclonal antibody has been shown to prevent IgE-mediated degranulation as well as *de novo* synthesis and release of interleukin 6. This molecule is therefore likely to be a negative regulator of mast cell/basophil functional effects exerted via the high affinity FcεR1 receptor. The negative effects of the rMAFA molecule on cell function are thought to originate from the cytoplasmic region of the molecule, which are the amino-terminal 34 amino acids. Within this region is a particular sequence [SEQ ID No. 23] (YSTL) containing a single copy of the motif YXXL/I [SEQ ID No. 24] found to be essential in immunoreceptor tyrosine activation motifs (ITAMS). However, the T-cell receptor (TCR), B-cell receptor (BCR) and FcεR1 are multi-subunit receptors which possess ten, four and three ITAMs respectively. Studies on the low affinity IgG receptor FcγRIIB have demonstrated that cell activation triggered by the aforementioned immunoreceptors can be inhibited if there is receptor coaggregation with FcγRIIB (Daëron *et al*, 1995; Muta *et al*. 1994). FcγRIIB has a single YXXL/I motif (similar to rat MAFA), responsible for the immunoreceptor inhibition, which is now known as an immunoreceptor tyrosine-based inhibition motif (ITIM). The ITIM mechanism of action *in vivo* is uncertain, however it is likely that the ITIM tyrosine residue is first phosphorylated by a src-like protein tyrosine kinase which allows the recruitment of an SH2-domain containing protein or lipid phosphatase which then acts on components of the immunoreceptor signalling cascade (Ono *et al*, 1996). Indeed, changes in the MAFA tyrosyl- and seryl-phosphorylation levels are observed in response to G63 binding, antigenic stimulation, and a combination of both treatments.

Summary of Invention

The rat MAFA molecule found on both mast cells and basophils has been cloned and shown to be a type II membrane glycoprotein with homology to calcium-dependent lectins. Alternatively spliced mRNA forms have been described, but the physiological relevance of these forms is unknown.

In this invention, we have cloned the human MAFA molecule. This molecule is similar to the rat form having an intracellular domain containing a putative ITIM motif and the extracellular lectin-like domain, however the amino acid sequence suggests the presence of two additional extracellular N-linked glycosylation sites.

Interestingly, alternative mRNA transcripts that are very different to the rat transcripts have been identified. Furthermore, a major transcript, not found in rat, but highly expressed in human lung and granulocyte-enriched blood cells, encodes a putative protein with the MAFA intracellular and transmembrane domains followed by an 8 amino acid polyproline motif due to a reading frameshift. This unique sequence has been used in the design of agents that can be used in the treatment of inflammation or allergy. Specifically, peptides of the generic amino acid sequence X-Pro-X-Pro-X-X-Pro [SEQ ID No. 1] were shown to inhibit both T cell antigen receptor-dependent activation induced interleukin 2 secretion from human Jurkat T cells and IgE-dependent degranulation of RBL cells. Interleukin 2 is an autocrine growth factor for T cells. Therefore inhibiting its production prevents T cell proliferation and hence suppresses the immune system.

The sequence of the human form of the MAFA molecule obtained from both the myelogenous leukaemic cell line KU812 and cDNA derived from human lung tissue is detailed in Fig. 1. Surprisingly, additional truncated forms of MAFA are provided which are expressed in the cells and tissues. One prominent form sequenced was found to encode a variant of the molecule in which the exon encoding the most N-terminal extracellular region (analogous to rat exon 3) was spliced out (huMAFA[E3-]). This phenomenon resulted in a coding amino acid frameshift, caused by the addition of an extra guanine nucleotide, resulting in truncation of the full length protein after the transmembrane domain. In addition, a new peptide motif of eight amino acids was encoded N-terminal to the new stop codon but continuous with the transmembrane sequence (fig. 2).

A third alternatively spliced huMAFA variant was identified (huMAFA[E3/4-]) which lacked the entire C-lectin-like domain but retained the intracellular and transmembrane domains as well as the extracellular C-terminal tail (fig 3).

Interestingly, both forms are membrane bound forms of MAFA. No soluble forms of MAFA were found corresponding to the rat MAFA [Exon 2-]

Previously, an inhibitory function has been proposed for rat MAFA (Guthmann *et al*, 1995). The co-aggregation of MAFA with cross-linked FcεR1 receptors, together with the suggestion that the ITIM motif may allow intracellular binding of a protein or inositol phosphatase, led to the hypothesis that MAFA may function as an "off" switch in regulating mast cell activation. This is accomplished by dephosphorylating the molecules of the FcεR1 complex or membrane lipids which become phosphorylated within seconds of receptor cross-linking. Although the extracellular receptor for MAFA is unknown, truncated versions of membrane-bound human MAFA could modulate the negative regulatory mechanisms. This is indicated by the results shown herein.

Therapies directed against the truncated forms of the molecule or its production would be expected to downregulate mast cell activation, and might therefore be useful in the treatment of allergic diseases. Similarly, overproduction of truncated MAFA may be associated with the development of atopy, and diagnosis of this could be accomplished using antibodies directed against unique C-terminal sequences expressed on the truncated form. Manipulation of the production and/or function of truncated MAFA are all encompassed within the scope of the invention.

In a first embodiment the invention provides a polypeptide which comprises or consists of the sequence of amino acid residues:

X-Pro-X-Pro-X-X-Pro. [SEQ ID No. 1]

Preferably it comprises or consists of the sequence of amino acid residues selected from the group:

Pro-Pro-Leu-Pro-Gln-X-Pro [SEQ ID No. 2]

Val-Pro-Val-Pro-Lys-X-Pro [SEQ ID No. 3]

Gly-Pro-Leu-Pro-Lys-X-Pro [SEQ ID No. 4]

Ala-Pro-Leu-Pro-His-X-Pro [SEQ ID No. 5]

Thr-Pro-Leu-Pro-Lys-X-Pro [SEQ ID No. 6]

Glu-Pro-Ala-Pro-Ser-Phe-Pro-Gln. [SEQ ID No. 7]

It may comprise or consists of the sequence of amino acid residues corresponding to human MAFA or a truncated form thereof. Preferably the truncated form is huMAFA[E3-] or huMAFA[E3/4-].

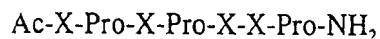
As used herein the term "a polypeptide which comprises or consists of the sequence of amino acid sequences" means either (i) a polypeptide which includes in its sequence the identified sequence "motif" as part of the polypeptide, or; (ii) a polypeptide which is terminated and has the sequence of the identified sequence motif.

For example, a polypeptide of type (i) is cloned human MAFA or a truncated version thereof which includes the motif sequence

7

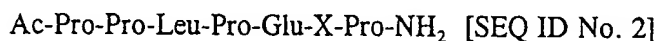
-X-Pro-X-Pro-X-X-Pro- [SEQ ID No. 1]

For example, a polypeptide of type (ii) is a peptide of formula



Although polypeptides according to the invention may contain an amino acid motif included in a relatively long sequence (such as full length human MAFA) this invention also provides relatively short length amino acid sequences of general formula $(aa)_n$ wherein n is any integer between 7 and 20, preferably between 7 and 10, most preferably 7 or 8.

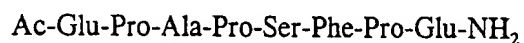
By way of example, the 7mer polypeptide of amino acid sequence



Consists of the motif sequence



whereas the 8mer polypeptide of sequence



includes the same motif.

In a second embodiment the invention provides a nucleotide sequence which codes for the polypeptide sequence.

In a third embodiment the invention provides an antibody or fragment thereof specific for an epitope of the C terminal extracellular domain sequences expressed on spliced type II C-lectin-like membrane proteins or an epitope of the N terminal intracellular domain sequences of type II C-lectin-like membrane proteins. Preferably the type II C-lectin-like membrane protein is human MAFA or a truncated form thereof. Preferably the truncated form is human MAFA[E3-] or human MAFA[E3/4-].

In a fourth embodiment the invention provides a ligand specific for a fragment of human MAFA which is expressed on the surface of filamentous phage.

In a fifth embodiment the invention provides a composition comprising a therapeutic amount of the polypeptide, antibody or fragment thereof or ligand, together with a pharmaceutically acceptable diluent or carrier.

In a sixth embodiment the invention provides the polypeptide, nucleotide sequence, antibody, or fragment thereof, ligand or composition for use as a medicament. Preferably they are used in the treatment of inflammatory or allergic diseases or tumour growth.

In a seventh embodiment the invention provides use of the polypeptide, nucleotide sequence, antibody or fragment thereof, ligand or composition in the manufacture of a medicament for the treatment of inflammatory or allergic diseases or tumour growth.

In an eighth embodiment the invention provides a method of treatment for inflammatory or allergic diseases which comprises administering an effective dose of the polypeptide, antibody or fragment thereof, ligand or composition.

In a ninth embodiment the invention provides a method of preparing the polypeptide which comprises the steps of:

- i) N α -Fmoc deprotection;
- ii) washing;
- iii) coupling of a single amino acid residue or amino acid mixtures;
- iv) washing;
- v) repeating until the desired polypeptide is constructed.

Detailed Description of the Invention

The invention will now be described by reference to the accompanying drawings in which:

Figure 1 shows the nucleotide sequence [SEQ ID No. 8,9] encoding the full-length expressed form of human MAFA (nucleotides 1-570). The expected amino acid translation is shown beneath the nucleotide sequence. Putative N-linked glycosylation sites are underlined. (The two amino acids in italics refer to polymorphic mutations)

Figure 2 shows the nucleotide sequence and putative amino acid sequence [SEQ ID No. 10,11] of 400 bp alternative human transcript (huMAFA[E3-]). Amino acid translation resulting from reading frame-shift is shown in bold. (* represents a stop codon so no further transcription occurs. Italic amino acids from polymorphic mutations)

Figure 3 shows the nucleotide sequence and putative amino acid sequence [SEQ ID No. 12,13] of 301 bp alternative human MAFA transcript (huMAFA[E3/4-]). The nucleotide sequence encoding the huMAFA C-terminal region is underlined (Analogous to rat Exon 5). (Italic amino acids from polymorphic mutations).

Figure 4 shows the nucleotide and amino acid sequence [SEQ ID No. 14,15] of rat MAFA. Putative N-linked glycosylation sites are underlined.

Blast program searches using the Internet NCBI software for human C-lectin-like sequences identified two expressed sequence tags (ESTs) AA186699 and AA188327 which showed some homology to the rat MAFA cDNA sequence. After careful analysis of the EST sequences, we designed PCR primers which we predicted represented the 5' and 3' end of the human MAFA coding cDNA. PCR using these primers on cDNA made from basophil-like leukaemic cells (KU812s), mast cell-enriched lung cells and basophil-enriched blood cells resulted in three different sized PCR DNA products of approximately 580, 400 and 300 bp. These DNA products were cloned into the sequencing vector pCR-script (Stratagene) and sequenced in both the forward and reverse directions using the T7 and T3 sequencing primers.

The largest PCR product was shown to represent the full coding sequence for human MAFA (fig. 1), a 400 bp product huMAFA[E3-] (fig. 2.) and a 300 bp product huMAFA[E3/4-] (fig. 3).

The full length human MAFA is one amino acid longer than its rat homologue and possesses two additional N-linked glycosylation sites (fig. 1). Two presumed polymorphic mutations were detected between samples of nucleotide 95 A→G resulting in a codon change of Lys to Arg and nucleotide 124 A→G resulting in a codon change of threonine to alanine. These changes are quite conservative and probably do not affect structure or function.

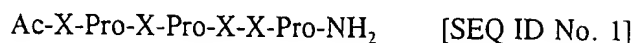
Sequences based on the alternatively spliced human MAFA[E3-] variant

The human MAFA[E3-] variant has the same putative intracellular and transmembrane amino acid sequence as full length MAFA, but following this sequence is the unique sequence:

Glu-Pro-Ala-Pro-Ser-Phe-Pro-Gln. [SEQ ID No. 7]

This sequence has been synthesised.

A library of peptides was constructed to represent the generic structure:



These library peptides were tested in whole cell systems for their ability to modulate the effects of cell stimulation. Separate peptide mixtures were found that could inhibit T-cell antigen receptor dependent interleukin-2 release from human T cells or prevent IgE-mediated degranulation of rat basophils:

Interleukin-2 Inhibitors are included in the following mixtures:

Ac-Pro-Pro-Leu-Pro-Gln-A/E/F/G/I/L/K/H/N/P/Q/S/T/V/Y-Pro-NH₂ [SEQ ID No. 16]

Ac-Gly-Pro-Leu-Pro-Lys-A/E/F/G/I/L/K/H/N/P/Q/S/T/V/Y-Pro-NH₂ [SEQ ID No. 17]

Ac-Val-Pro-Val-Pro-Lys-A/E/F/G/I/L/K/H/N/P/Q/S/T/V/Y-Pro-NH₂ [SEQ ID No. 18]

Degranulation Inhibitors are included in the following mixtures:

Ac-Ala-Pro-Leu-Pro-His-A/E/F/G/I/L/K/H/N/P/Q/S/T/V/Y-Pro-NH₂ [SEQ ID No. 19]

Ac-Thr-Pro-Leu-Pro-Lys-A/E/F/G/I/L/K/H/N/P/Q/S/T/V/Y-Pro-NH₂ [SEQ ID No. 20]

These sequences indicate that the generic sequences for interleukin-2 are:

Pro-Pro-Leu-Pro-Gln-X-Pro

Val-Pro-Val-Pro-Lys-X-Pro

Gly-Pro-Leu-Pro-Lys-X-Pro

and the generic sequences for degranulation inhibitors are:

Ala-Pro-Leu-Pro-His-X-Pro

Thr-Pro-Leu-Pro-Lys-X-Pro

Experimental Methods

Cell Culture

Ku 812 cells (Kishi, 1985) and Jurkat E17 T cells (Williams *et al*, 1995) were grown in RPMI 1640 (GIBCO) supplemented with 10 % (vol/vol) heat inactivated foetal calf serum, 50 IU/ml penicillin, 50 µg/ml streptomycin and 2 mM L-glutamine. RBL cells were grown in DMEM (GIBCO) supplemented with 10 % (vol/vol) heat inactivated foetal calf serum, 50 IU/ml penicillin, 50 µg/ml streptomycin. Growth of all cells was at 37°C in humidified 5 % CO₂/95 % air.

Cell Isolation

Peripheral blood cells obtained in "buffy" coats were fractionated using Ficoll® and washed white cell pellet further fractionated using Percoll as described by Raghuprasad (1982) to provide basophil-enriched cell populations. Red blood cells were lysed by suspending cell pellet twice in 8.29 g/l NH₄Cl, 0.84 g/l NaHCO₃, 37.3 µg/l EDTA, pH 7.3. Remaining cells were treated as basophil-enriched cells and shown to contain 10-20% basophils, after Wright's solution staining of cytopsin prepared slide samples..

Human lung biopsy samples (100-170 g) were minced finely using scissors and placed in enzymatic digestion cocktail (35 mg/ml BSA, fraction V, 0.38 mg/ml Hyaluronidase, 0.25

mg/ml Pronase, 0.03 mg/ml Dnase I, 0.75 mg/ml bacterial collagenase in DMEM) at 5 ml cocktail/g tissue for 1 hour at 37°C with agitation. The digest was then filtered through a 0.75µm nylon mesh to remove undigested material and washed twice in PBS. Samples of these cells were stained using Wrights solution and found to contain 5-10% mast cells.

RBL degranulation assays

RBL cells were harvested by scraping and resuspended to 1×10^6 cells/ml in DMEM supplemented with 10 % (vol/vol) heat inactivated foetal calf serum, 50 IU/ml penicillin, 50 µg/ml streptomycin. 50 µl cells were added to wells in a 96 well flat-bottomed plate and incubated overnight at 37°C with 50 µl 200 ng/ml anti DNP-IgE. The medium was then replaced with degranulation buffer (phenol red free RPMI 1640 (Gibco), 1g glucose , 0.5g BSA in 500 ml) containing 4 µM test peptide and 37°C incubation performed for 1 hour. DNP-BSA was then added to 100 ng/ml and incubation performed for a further 45 minutes. Buffer was then removed from the cells and assayed for hexosaminidase activity.

Interleukin 2 secretion assay

Jurkat E17 T cells were harvested from actively growing suspension culture and resuspended to 4×10^6 cells/ml in fresh medium. Cells were plated out in 96 well plates with test peptide at 2 µM at a concentration of 2×10^6 cells/ml. Precubation was then performed for 1 hour at 37°C followed by the addition of 2 µg/ml PHA and 50 ng/ml PDBu and overnight 37°C incubation. Medium was then removed and assayed to determine the amount of interleukin 2 by ELISA (Genzyme kit).

Reverse Transcribed-Polymerase Chain Reactions (RT-PCR)

Messenger RNA (mRNA) was isolated cell pellets using a Pharmacia mRNA isolation kit, as described in manufacturers instructions, this was used to make cDNA utilising oligo dT primers. Single-stranded DNA 5' and 3' primers were designed to amplify the full human

MAFA coding sequence flanked by Bam H1 restriction enzyme sites.

5' primer;

GCCGGATCCGATGACTGACAGTGTTATTTATTCCATGTTA [SEQ ID No. 21]

3' primer;

TAAGGATCCTCAAAGTCTGACCTTCTTACACACCCAGTG [SEQ ID No. 22]

PCR using these primers and 20 ng template cDNA was performed at 94°C, 2 minutes, then 35 cycles of 94°C for 15 seconds, 65°C for 30 seconds and 72°C for 45 seconds followed by 72°C for 5 minutes using KlenTaq (Clontech, USA). PCR amplicons were then cloned into pCR-script (Stratagene) as described in manufacturers instructions prior to DNA sequencing on an applied biosystems DNA sequencer.

DNA sequence analysis

DNA sequencing was performed using the Perkin-Elmer Taq polymerase system in conjunction with an Applied Biosystems 373 sequencer. Sequence analysis was performed using DNASTar and NCBI blast programs.

Peptide Synthesis

Peptides were prepared by the multipin synthesis technique which is set out below:

Preparation of Multipin Assembly

Whilst wearing standard plastic gloves, the Fmoc-Rink-DA/MDA macrocrowns are assembled (simply clipped) onto stems and slotted into a 8 x 12 stem holder in the desired pattern for synthesis.

Peptides are then prepared as singles or defined equimolar mixtures by repetitive rounds of

N α -Fmoc deprotection, washing, coupling of a single aminoacid or aminoacid mixtures, washing etc until the desired primary sequences have been constructed.

Removal of N α -Fmoc Protection

A 250 ml solvent resistant bath was charged with 200 ml of a 20% piperidine/DMF solution. The multipin assembly was added and deprotection allowed to proceed for 30 minutes. The assembly was then removed and excess solvent removed by brief shaking. The assembly is then washed consecutively with (200 ml each), DMF (5 minutes) and MeOH (5 minutes, 2 minutes, 2 minutes) and left to air dry for 15 minutes.

Quantitative UV Measurement of Fmoc Chromophore Release

A 1 cm path length UV cell was charged with 1.2 ml of a 20% piperidine/DMF solution and used to zero the absorbance of the UV spectrometer at a wavelength of 290nm. A UV standard was then prepared consisting of 5.0 mg Fmoc-Asp(OBut)-Pepsyn KA (0.08 mmol/g) in 3.2 ml of a 20% piperidine/DMF solution. This standard gives $Abs_{290} = 0.55-0.65$ (at room temperature). An aliquot of the multipin deprotection solution was then diluted as appropriate to give a theoretical $Abs_{290} = 0.6$, and this value compared with the actual experimentally measured absorbance showing the efficiency of previous coupling reaction.

Coupling of Standard Amino Acid Residues

Coupling reactions were performed by charging the appropriate wells of a polypropylene 96 well plate with the pattern of activated solutions required during a particular round of coupling. MacroCrown (approx 7 μ mole) standard couplings were performed in DMF (500 μ l).

Coupling of an Amino-acid Residue To Appropriate Well

Whilst the multipin assembly was drying, the appropriate N α -Fmoc amino acid pfp esters (10 equivalents calculated from the loading of each crown) and HOBt (10 equivalents) required for the particular round of coupling are accurately weighed into suitable containers. Alternatively, the appropriate N α -Fmoc amino acids (10 equivalents calculated from the loading of each crown), desired coupling agent e.g. HBTU (9.9 equivalents calculated from the loading of each crown) and activation e.g. HOBt (9.9 equivalents calculated from the loading of each crown), NMM (19.9 equivalents calculated from the loading of each crown) are accurately weighed into suitable containers.

The protected and activated Fmoc amino acid derivatives are then dissolved in DMF (500 μ l for each macrocrown e.g. for 20 macrocrowns, 20 x 10 eq. x 7 μ moles of derivative would be dissolved in 10 000 μ l DMF). The appropriate derivatives were then dispensed to the appropriate wells ready for commencement of the 'coupling cycle'. As a standard, coupling reactions were allowed to proceed for 6 hours. The coupled assembly was then washed as detailed below.

Equimolar Coupling Of An Amino Acid Residue Mixture

Equimolar coupling reactions were performed by charging the appropriate wells of a polypropylene 96 well plate with the pattern of activated solutions required during a particular round of coupling. The equimolar coupling cycle is a 3 stage cycle consisting of:-

0.98eq coupling overnight, i.e. for the equimolar addition of 15 residues, $0.98 / 15 = 0.0653\text{eq}$ of each residue is weighed and activated as a single mixture.

Repeat of 1)

A 9.8eq coupling for 3hrs, i.e. for the equimolar addition of 15 residues, $9.8 / 15 = 0.653\text{eq}$ of each residue is weighed and activated as a single mixture

Equimolar Coupling of an Amino-acid Residue Mixture To Appropriate Well

Whilst the multipin assembly was drying, the appropriate $N\alpha$ -Fmoc amino acid pfp esters and HOBT required for the particular round of equimolar coupling were accurately weighed into suitable containers (see above for mixture composition). Alternatively, the appropriate $N\alpha$ -Fmoc amino acids, desired coupling agent e.g. HBTU and activation e.g. HOBT, NMM were accurately weighed into suitable containers (see above for mixture composition).

The protected and activated Fmoc amino acid derivatives are then dissolved in DMF (500 μl for each macrocrown e.g. for 20 macrocrowns, $20 \times 10 \text{ eq.} \times 7 \mu\text{moles of derivative}$ was dissolved in 10 000 μl DMF). The appropriate derivatives were then dispensed to the appropriate wells ready for commencement of the 'coupling cycle'. The standard equimolar coupling procedure is outlined above. The coupled assembly was then washed as detailed below.

Washing Following Coupling

If a 20% piperidine/DMF deprotection was to immediately follow the coupling cycle, then the multipin assembly was briefly shaken to remove excess solvent washed consecutively with (200 ml each), MeOH (5 minutes) and DMF (5 minutes) and de-protected (see 6.2). If the multipin assembly was to be stored or reacted further, then a full washing cycle consisting brief shaking then consecutive washes with (200 ml each), DMF (5 minutes) and MeOH (5 minutes, 2 minutes, 2 minutes) was performed.

Acidolytic Mediated Cleavage of Peptide-Pin Assembly

Acid mediated cleavage protocols were strictly performed in a fume hood. A polystyrene 96 well plate (1 ml/well) was labelled, then the tare weight measured to the nearest mg.

Appropriate wells were then charged with a trifluoroacetic acid/triethylsilane (95:5, v/v, 600 μ l) cleavage solution, in a pattern corresponding to that of the multipin assembly to be cleaved.

The multipin assembly was added, the entire construct covered in tin foil and left for 2 hours. The multipin assembly was then added to another polystyrene 96 well plate (1 ml/well) containing trifluoroacetic acid/triethylsilane (95:5, v/v, 600 μ l) (as above) for 5 minutes.

Work up of Cleaved Peptides

The primary polystyrene cleavage plate (2 hour cleavage) and the secondary polystyrene plate (5 minute wash) were then placed in the SpeedVac and the solvents removed (minimum drying rate) for 90 minutes.

The contents of the secondary polystyrene plate were transferred to their corresponding wells on the primary plate using an acetonitrile/water/acetic acid (50:45:5, v/v/v) solution (3 x 150 μ l) and the spent secondary plate discarded.

Analysis of Products

A 5 μ L aliquot from each well was diluted to 100 μ l with 0.1% aq. TFA, then a 10 μ L aliquot from this plate diluted with a further 100 μ l 0.1% aq. TFA. The double diluted plate was analysed by HPLC-MS.

Final Lyophilisation of Peptides

The plate was covered with tin foil, held to the plate with an elastic band. A pin prick was placed in the foil directly above each well and the plate placed at -80°C for 30 minutes. The plate was then lyophilised on the 'Heto freeze drier' overnight.

Finally, the dried plate was weighed. The total cleaved peptide was quantified (by weight) and the average content of each peptide calculated. Since all the peptides present originated from the same peptide-pin assembly, cleaved under identical conditions, it is reasonable to assume that the contents of each well were approximately equimolar.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: PEPTIDE THERAPEUTICS LIMITED
- (B) STREET: 321 CAMBRIDGE SCIENCE PARK
- (C) CITY: CAMBRIDGE
- (D) STATE: CAMBRIDGE
- (E) COUNTRY: ENGLAND
- (F) POSTAL CODE (ZIP): CB4 4WG
- (G) TELEPHONE: 01223 423333
- (H) TELEFAX: 01223 423111

(ii) TITLE OF INVENTION: Human MAFA

(iii) NUMBER OF SEQUENCES: 24

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Xaa Pro Xaa Pro Xaa Xaa Pro
1 5

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Pro Pro Leu Pro Gln Xaa Pro
1 5

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Val Pro Val Pro Lys Xaa Pro
1 5

(2) INFORMATION FOR SEQ ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Gly Pro Leu Pro Lys Xaa Pro
1 5

(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ala Pro Leu Pro His Xaa Pro
1 5

(2) INFORMATION FOR SEQ ID NO: 6:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Thr Pro Leu Pro Lys Xaa Pro
1 5

(2) INFORMATION FOR SEQ ID NO: 7:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Glu Pro Ala Pro Ser Phe Pro Gln
1 5

(2) INFORMATION FOR SEQ ID NO: 8:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 570 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION:1..567

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

ATG ACT GAC AGT GTT ATT TAT TCC ATG TTA GAG TTG CCT ACG GCA ACC	48
Met Thr Asp Ser Val Ile Tyr Ser Met Leu Glu Leu Pro Thr Ala Thr	
1 5 10 15	
CAA GCC CAG AAT GAC TAC GGA CCA CAG CAA AAA TCT TCC TCT TCC AAG	96
Gln Ala Gln Asn Asp Tyr Gly Pro Gln Gln Lys Ser Ser Ser Ser Lys	
20 25 30	
CCT TCT TGT TCT TGC CTT GTG GCA ATA ACT TTG GGG CTT CTG ACT GCA	144
Pro Ser Cys Ser Cys Leu Val Ala Ile Thr Leu Gly Leu Leu Thr Ala	
35 40 45	
GTT CTT CTG AGT GTG CTG CTA TAC CAG TGG ATC CTG TGC CAG GGC TCC	192
Val Leu Leu Ser Val Leu Leu Tyr Gln Trp Ile Leu Cys Gln Gly Ser	
50 55 60	
AAC TAC TCC ACT TGT GCC AGC TGT CCT AGC TGC CCA GAC CGC TGG ATG	240
Asn Tyr Ser Thr Cys Ala Ser Cys Pro Ser Cys Pro Asp Arg Trp Met	
65 70 75 80	
AAA TAT GGT AAC CAT TGT TAT TAT TTC TCA GTG GAG GAA AAG GAC TGG	288
Lys Tyr Gly Asn His Cys Tyr Tyr Phe Ser Val Glu Glu Lys Asp Trp	
85 90 95	
AAT TCT AGT CTG GAA TTC TGC CTA GCC AGA GAC TCA CAC CTC CTT GTG	336
Asn Ser Ser Leu Glu Phe Cys Leu Ala Arg Asp Ser His Leu Leu Val	
100 105 110	
ATA ACG GAC AAT CAG GAA ATG AGC CTG CTC CAA GTT TTC CTC AGT GAG	384
Ile Thr Asp Asn Gln Glu Met Ser Leu Leu Gln Val Phe Leu Ser Glu	
115 120 125	
GCC TTT TGC TGG ATT GGT CTG AGG AAC AAT TCT GGC TGG AGG TGG GAA	432
Ala Phe Cys Trp Ile Gly Leu Arg Asn Asn Ser Gly Trp Arg Trp Glu	
130 135 140	
GAC GGA TCA CCT CTA AAC TTC TCA AGG ATT TCT TCT AAT AGC TTT GTG	480
Asp Gly Ser Pro Leu Asn Phe Ser Arg Ile Ser Ser Asn Ser Phe Val	
145 150 155 160	
CAG ACA TGC GGT GCC ATC AAC AAA AAT GGT CTT CAA GCC TCA AGC TGT	528
Gln Thr Cys Gly Ala Ile Asn Lys Asn Gly Leu Gln Ala Ser Ser Cys	
165 170 175	
GAA GTT CCT TTA CAC GGG GTG TGT AAG AAG GTC AGA CTT TGA	570
Glu Val Pro Leu His Gly Val Cys Lys Lys Val Arg Leu	
180 185	

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

24

- (A) LENGTH: 189 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```

Met Thr Asp Ser Val Ile Tyr Ser Met Leu Glu Leu Pro Thr Ala Thr
 1           5           10           15

Gln Ala Gln Asn Asp Tyr Gly Pro Gln Gln Lys Ser Ser Ser Ser Lys
          20           25           30

Pro Ser Cys Ser Cys Leu Val Ala Ile Thr Leu Gly Leu Leu Thr Ala
          35           40           45

Val Leu Leu Ser Val Leu Leu Tyr Gln Trp Ile Leu Cys Gln Gly Ser
 50           55           60

Asn Tyr Ser Thr Cys Ala Ser Cys Pro Ser Cys Pro Asp Arg Trp Met
 65           70           75           80

Lys Tyr Gly Asn His Cys Tyr Tyr Phe Ser Val Glu Glu Lys Asp Trp
          85           90           95

Asn Ser Ser Leu Glu Phe Cys Leu Ala Arg Asp Ser His Leu Leu Val
        100           105           110

Ile Thr Asp Asn Gln Glu Met Ser Leu Leu Gln Val Phe Leu Ser Glu
        115           120           125

Ala Phe Cys Trp Ile Gly Leu Arg Asn Asn Ser Gly Trp Arg Trp Glu
        130           135           140

Asp Gly Ser Pro Leu Asn Phe Ser Arg Ile Ser Ser Asn Ser Phe Val
        145           150           155           160

Gln Thr Cys Gly Ala Ile Asn Lys Asn Gly Leu Gln Ala Ser Ser Cys
          165           170           175

Glu Val Pro Leu His Gly Val Cys Lys Lys Val Arg Leu
        180           185

```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..210

(xi) SEQUENCE DESCRIPTION: SEO ID NO: 10:

[illegible]

(2) INFORMATION FOR SEO ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEO ID NO: 11:

```

Met Thr Asp Ser Val Ile Tyr Ser Met Leu Glu Leu Pro Thr Ala Thr
  1                      5                      10                      15

Gln Ala Gln Asn Asp Tyr Gly Pro Gln Gln Lys Ser Ser Ser Ser Arg
      20                      25                      30

Pro Ser Cys Ser Cys Leu Val Ala Ile Ala Leu Gly Leu Leu Thr Ala
      35                      40                      45

Val Leu Leu Ser Val Leu Leu Tyr Gln Trp Ile Leu Cys Gln Glu Pro
      50                      55                      60

```


27

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```

Met Thr Asp Ser Val Ile Tyr Ser Met Leu Glu Leu Pro Thr Ala Thr
 1           5           10           15
Gln Ala Gln Asn Asp Tyr Gly Pro Gln Gln Lys Ser Ser Ser Ser Arg
      20           25           30
Pro Ser Cys Ser Cys Leu Val Ala Ile Ala Leu Gly Leu Leu Thr Ala
      35           40           45
Val Leu Leu Ser Val Leu Leu Tyr Gln Trp Ile Leu Cys Gln Gly Ile
      50           55           60
Ser Ser Asn Ser Phe Val Gln Thr Cys Gly Ala Ile Thr Lys Asn Gly
      65           70           75           80
Leu Gln Ala Ser Ser Cys Glu Val Pro Leu His Trp Val Cys Lys Lys
      85           90           95
Val Arg Leu

```

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 567 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION:1..564

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

```

ATG GCC GAC AAC TCT ATC TAC TCA ACA TTA GAG CTG CCT GCT GCA CCT      48
Met Ala Asp Asn Ser Ile Tyr Ser Thr Leu Glu Leu Pro Ala Ala Pro
100           105           110           115
CGA GTC CAA GAT GAC TCC AGA TGG AAG GTC AAA GCT GTC TTA CAC CGA      96
Arg Val Gln Asp Asp Ser Arg Trp Lys Val Lys Ala Val Leu His Arg
      120           125           130
CCC TGT GTT TCC TAC CTT GTG ATG GTG GCT TTG GGG CTT TTG ACT GTG      144
Pro Cys Val Ser Tyr Leu Val Met Val Ala Leu Gly Leu Leu Thr Val
      135           140           145

```

28

ATT CTC ATG AGT CTA CTG TTG TAC CAA CGG ACT CTG TGC TGT GGC TCC Ile Leu Met Ser Leu Leu Leu Tyr Gln Arg Thr Leu Cys Cys Gly Ser 150 155 160	192
AAG GGC TTT ATG TGT TCC CAG TGC TCC AGG TGC CCC AAC CTC TGG ATG Lys Gly Phe Met Cys Ser Gln Cys Ser Arg Cys Pro Asn Leu Trp Met 165 170 175	240
AGG AAC GGG AGC CAC TGT TAC TAC TTC TCA ATG GAG AAA AGG GAC TGG Arg Asn Gly Ser His Cys Tyr Tyr Phe Ser Met Glu Lys Arg Asp Trp 180 185 190 195	288
AAC TCT AGT CTG AAG TTC TGT GCA GAC AAA GGC TCG CAT CTC CTT ACA Asn Ser Ser Leu Lys Phe Cys Ala Asp Lys Gly Ser His Leu Leu Thr 200 205 210	336
TTT CCG GAC AAC CAG GGA GTG AAC CTG TTC CAG GAG TAT GTG GGC GAG Phe Pro Asp Asn Gln Gly Val Asn Leu Phe Gln Glu Tyr Val Gly Glu 215 220 225	384
GAC TTT TAC TGG ATT GGC TTG AGG GAC ATC GAT GGC TGG AGG TGG GAA Asp Phe Tyr Trp Ile Gly Leu Arg Asp Ile Asp Gly Trp Arg Trp Glu 230 235 240	432
GAT GGC CCA GCT CTC AGC TTA AGC ATT CTC TCT AAC AGC GTG GTA CAG Asp Gly Pro Ala Leu Ser Leu Ser Ile Leu Ser Asn Ser Val Val Gln 245 250 255	480
AAG TGT GGC ACC ATC CAC AGG TGT GGC CTC CAC GCC TCC AGT TGT GAG Lys Cys Gly Thr Ile His Arg Cys Gly Leu His Ala Ser Ser Cys Glu 260 265 270 275	528
GTT GCT TTG CAG TGG ATC TGT GAG AAG GTC CTG CCC TGA Val Ala Leu Gln Trp Ile Cys Glu Lys Val Leu Pro 280 285	567

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 188 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Met Ala Asp Asn Ser Ile Tyr Ser Thr Leu Glu Leu Pro Ala Ala Pro 1 5 10 15
Arg Val Gln Asp Asp Ser Arg Trp Lys Val Lys Ala Val Leu His Arg 20 25 30
Pro Cys Val Ser Tyr Leu Val Met Val Ala Leu Gly Leu Leu Thr Val 35 40 45

29

```

Ile Leu Met Ser Leu Leu Leu Tyr Gln Arg Thr Leu Cys Cys Gly Ser
  50                      55                      60

Lys Gly Phe Met Cys Ser Gln Cys Ser Arg Cys Pro Asn Leu Trp Met
  65                      70                      75                      80

Arg Asn Gly Ser His Cys Tyr Tyr Phe Ser Met Glu Lys Arg Asp Trp
                      85                      90                      95

Asn Ser Ser Leu Lys Phe Cys Ala Asp Lys Gly Ser His Leu Leu Thr
      100                      105                      110

Phe Pro Asp Asn Gln Gly Val Asn Leu Phe Gln Glu Tyr Val Gly Glu
      115                      120                      125

Asp Phe Tyr Trp Ile Gly Leu Arg Asp Ile Asp Gly Trp Arg Trp Glu
      130                      135                      140

Asp Gly Pro Ala Leu Ser Leu Ser Ile Leu Ser Asn Ser Val Val Gln
      145                      150                      155                      160

Lys Cys Gly Thr Ile His Arg Cys Gly Leu His Ala Ser Ser Cys Glu
      165                      170                      175

Val Ala Leu Gln Trp Ile Cys Glu Lys Val Leu Pro
      180                      185

```

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /note= "Xaa at position 6 is selected from the group which comprises A, E, F, G, I, L, K, H, N, P, Q, S, T, V, Y."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```

Pro Pro Leu Pro Gln Xaa Pro
  1                      5

```

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids

- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION:6
- (D) OTHER INFORMATION:/note= "Xaa at postion 6 is
selected from the group which comprises
A,E,F,G,I,L,K,H,N,P,Q,S,T,V,Y."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Gly Pro Leu Pro Lys Xaa Pro
1 5

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION:6
- (D) OTHER INFORMATION:/note= "Xaa at position 6 is
selected from the group which comprises AEEGILKHNPQSTVY"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Val Pro Val Pro Lys Xaa Pro
1 5

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

31

- (A) NAME/KEY: Modified-site
- (B) LOCATION:6
- (D) OTHER INFORMATION:/note= "Xaa at postion 6 is
selected from the group which comprises
A,E,F,G,I,L,K,H,N,P,Q,S,T,V,Y."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Ala Pro Leu Pro His Xaa Pro
1 5

(2) INFORMATION FOR SEQ ID NO: 20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 7 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION:6
- (D) OTHER INFORMATION:/note= "Xaa at position 6 is
selected from the group which comprises
A,E,F,G,I,L,K,H,N,P,Q,S,T,V,Y."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Thr Pro Leu Pro Lys Xaa Pro
1 5

(2) INFORMATION FOR SEQ ID NO: 21:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

GCCGGATCCG ATGACTGACA GTGTTATTTA TTCCATGTTA

40

(2) INFORMATION FOR SEQ ID NO: 22:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 39 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA to mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

TAAGGATCCT CAAAGTCTGA CCTTCTTACA CACCCAGTG

39

- (2) INFORMATION FOR SEQ ID NO: 23:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 4 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Tyr Ser Thr Leu
1

- (2) INFORMATION FOR SEQ ID NO: 24:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

- ```
(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION:4
 (D) OTHER INFORMATION:/note= "Xaa at position 4 is
 selected from the group which comprises L and..."
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Tyr Xaa Xaa Xaa  
1

## References

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Williams, D.H., Woodrow, M., Cantrell, D.A. and Murray, E.J. (1995). Protein kinase C is not a downstream effector of p21<sup>ras</sup> in activated T cells. *Eur. J. Immunol.*, **25**, 42-47.

Claims

1. A polypeptide which comprises or consists of the sequence of amino acid residues:

X-Pro-X-Pro-X-X-Pro.

2. A polypeptide according to claim 1 which comprises or consists of the sequence of amino acid residues selected from the group:

Pro-Pro-Leu-Pro-Gln-X-Pro

Val-Pro-Val-Pro-Lys-X-Pro

Gly-Pro-Leu-Pro-Lys-X-Pro

Ala-Pro-Leu-Pro-His-X-Pro

Thr-Pro-Leu-Pro-Lys-X-Pro

Glu-Pro-Ala-Pro-Ser-Phe-Pro-Gln.

3. A polypeptide according to claim 1 or 2 which consists of a sequence of 7 to 20, preferably 7 to 10, more preferably 7 or 8 amino acid residues.
4. A polypeptide which comprises or consists of the sequence of amino acid residues corresponding to a truncated form of human MAFA.
5. A polypeptide according to claim 4 wherein the truncated human MAFA is

huMAFA[E3-] or huMAFA[E3/4-].

6. A polypeptide which comprises or consists of the sequence of amino acid residues corresponding to human MAFA.
7. A nucleotide sequence which codes for the polypeptide sequence of any one of claims 1 to 6.
8. An antibody or fragment thereof specific for an epitope of the C terminal extracellular domain sequences expressed on spliced type II C-lectin-like membrane proteins.
9. An antibody or fragment thereof specific for an epitope of the N terminal intracellular domain sequences of type II C-lectin-like membrane proteins.
10. An antibody or fragment thereof according to claim 8 or 9 wherein the type II C-lectin-like membrane protein is human MAFA.
11. An antibody or fragment thereof according to claim 8, 9 or 10 wherein the protein is human MAFA[E3-] or human MAFA[E3/4-].
12. A ligand specific for a fragment of human MAFA which is expressed on the surface of filamentous phage.
13. A composition comprising a therapeutic amount of a polypeptide of claims 1 to 6; antibody or fragment thereof of claims 8 to 11; or ligand of claim 12, together with a pharmaceutically acceptable diluent or carrier.

14. A polypeptide according to any one of claims 1 to 6 for use as a medicament.
15. A polypeptide according to any one of claims 1 to 6 for use in the treatment of inflammatory or allergic diseases or tumour growth.
16. A nucleotide sequence according to claim 7 for use in therapy.
17. A nucleotide sequence according to claim 7 for use in the treatment of inflammatory or allergic diseases or tumour growth.
18. An antibody or fragment thereof according to any one of claims 8 to 11 for use as a medicament.
19. An antibody or fragment thereof according to any one of claims 8 to 11 for use in the treatment of inflammatory or allergic diseases or tumour growth.
20. A ligand according to claim 12 for use as a medicament.
21. A ligand according to claim 12 for use in the treatment of inflammatory or allergic diseases or tumour growth.
22. A composition according to claim 13 for use as a medicament.
23. A composition according to claim 13 for use in the treatment of inflammatory or allergic diseases or tumour growth.
24. Use of a polypeptide according to any one of claim 1 to 6 in the manufacture of a medicament for the treatment of inflammatory or allergic diseases or tumour growth.

25. Use of a nucleotide sequence according to claim 7 in the manufacture of a medicament for the treatment of inflammatory or allergic diseases or tumour growth.
26. Use of an antibody or fragment thereof according to any one of claims 8 to 11 in the manufacture of a medicament for the treatment of inflammatory or allergic diseases or tumour growth.
27. Use of a ligand according to claim 12 in the manufacture of a medicament for the treatment of inflammatory or allergic diseases or tumour growth.
28. Use of a composition according to claim 13 in the manufacture of a medicament for the treatment of inflammatory or allergic diseases or tumour growth.
29. A method of treatment for inflammatory or allergic diseases or tumour growth which comprises administering an effective dose of a polypeptide of claims 1 to 6; an antibody or fragment thereof of claims 8 to 11; a ligand of claim 12; or a composition of claim 13.
30. A method of preparing a polypeptide according to any one of claims 1 to 6 which comprises the steps of:
- i) N $\alpha$ -Fmoc deprotection;
  - ii) washing;
  - iii) coupling of a single amino acid residue or amino acid mixtures;
  - iv) washing;
  - v) repeating until the desired polypeptide is constructed.



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Figure 1

|     |          |          |          |          |          |          |          |          |          |     |     |     |     |     |          |
|-----|----------|----------|----------|----------|----------|----------|----------|----------|----------|-----|-----|-----|-----|-----|----------|
| 1   | ATG      | ACT      | GAC      | AGT      | GTT      | ATT      | TAT      | TCC      | ATG      | TTA | GAG | TTG | CCT | ACG | GCA      |
| 1   | M        | T        | D        | S        | V        | I        | Y        | S        | M        | L   | E   | L   | P   | T   | A        |
| 46  | ACC      | CAA      | GCC      | CAG      | AAT      | GAC      | TAC      | GGA      | CCA      | CAG | CAA | AAA | TCT | TCC | TCT      |
| 16  | T        | Q        | A        | Q        | N        | D        | Y        | G        | P        | Q   | Q   | K   | S   | S   | S        |
| 91  | TCC      | AAG      | CCT      | TCT      | TGT      | TCT      | TGC      | CTT      | GTG      | GCA | ATA | ACT | TTG | GGG | CTT      |
| 31  | S        | K        | P        | S        | C        | S        | C        | L        | V        | A   | I   | T   | L   | G   | L        |
| 136 | CTG      | ACT      | GCA      | GTT      | CTT      | CTG      | AGT      | GTG      | CTG      | CTA | TAC | CAG | TGG | ATC | CTG      |
| 46  | L        | T        | A        | V        | L        | L        | S        | V        | L        | L   | Y   | Q   | W   | I   | L        |
| 181 | TGC      | CAG      | GGC      | TCC      | AAC      | TAC      | TCC      | ACT      | TGT      | GCC | AGC | TGT | CCT | AGC | TGC      |
| 61  | C        | Q        | G        | S        | <u>N</u> | <u>Y</u> | <u>S</u> | T        | C        | A   | S   | C   | P   | S   | C        |
| 226 | CCA      | GAC      | CGC      | TGG      | ATG      | AAA      | TAT      | GGT      | AAC      | CAT | TGT | TAT | TAT | TTC | TCA      |
| 76  | P        | D        | R        | W        | M        | K        | Y        | G        | N        | H   | C   | Y   | Y   | F   | S        |
| 271 | GTG      | GAG      | GAA      | AAG      | GAC      | TGG      | AAT      | TCT      | AGT      | CTG | GAA | TTC | TGC | CTA | GCC      |
| 91  | V        | E        | E        | K        | D        | W        | <u>N</u> | <u>S</u> | <u>S</u> | L   | E   | F   | C   | L   | A        |
| 316 | AGA      | GAC      | TCA      | CAC      | CTC      | CTT      | GTG      | ATA      | ACG      | GAC | AAT | CAG | GAA | ATG | AGC      |
| 106 | R        | D        | S        | H        | L        | L        | V        | I        | T        | D   | N   | Q   | E   | M   | S        |
| 361 | CTG      | CTC      | CAA      | GTT      | TTC      | CTC      | AGT      | GAG      | GCC      | TTT | TGC | TGG | ATT | GGT | CTG      |
| 121 | L        | L        | Q        | V        | F        | L        | S        | E        | A        | F   | C   | W   | I   | G   | L        |
| 406 | AGG      | AAC      | AAT      | TCT      | GGC      | TGG      | AGG      | TGG      | GAA      | GAC | GGA | TCA | CCT | CTA | AAC      |
| 136 | R        | <u>N</u> | <u>N</u> | <u>S</u> | <u>G</u> | W        | R        | W        | E        | D   | G   | S   | P   | L   | <u>N</u> |
| 451 | TTC      | TCA      | AGG      | ATT      | TCT      | TCT      | AAT      | AGC      | TTT      | GTG | CAG | ACA | TGC | GGT | GCC      |
| 151 | <u>F</u> | <u>S</u> | R        | I        | S        | S        | N        | S        | F        | V   | Q   | T   | C   | G   | A        |
| 496 | ATC      | AAC      | AAA      | AAT      | GGT      | CTT      | CAA      | GCC      | TCA      | AGC | TGT | GAA | GTT | CCT | TTA      |
| 166 | I        | N        | K        | N        | G        | L        | Q        | A        | S        | S   | C   | E   | V   | P   | L        |
| 541 | CAC      | GGG      | GTG      | TGT      | AAG      | AAG      | GTC      | AGA      | CTT      | TGA |     |     |     |     |          |
| 181 | H        | G        | V        | C        | K        | K        | V        | R        | L        | *   |     |     |     |     |          |

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Figure 2

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   | ATG | ACT | GAC | AGT | GTT | ATT | TAT | TCC | ATG | TTA | GAG | TTG | CCT | ACG | GCA |
| 1   | M   | T   | D   | S   | V   | I   | Y   | S   | M   | L   | E   | L   | P   | T   | A   |
| 46  | ACC | CAA | GCC | CAG | AAT | GAC | TAC | GGA | CCA | CAG | CAA | AAA | TCT | TCC | TCT |
| 16  | T   | Q   | A   | Q   | N   | D   | Y   | G   | P   | Q   | Q   | K   | S   | S   | S   |
| 91  | TCC | AGG | CCT | TCT | TGT | TCT | TGC | CTT | GTG | GCA | ATA | GCT | TTG | GGG | CTT |
| 31  | S   | R   | P   | S   | C   | S   | C   | L   | V   | A   | I   | A   | L   | G   | L   |
| 136 | CTG | ACT | GCA | GTT | CTT | CTG | AGT | GTG | CTG | CTA | TAC | CAG | TGG | ATC | CTG |
| 46  | L   | T   | A   | V   | L   | L   | S   | V   | L   | L   | Y   | Q   | W   | I   | L   |
| 181 | TGC | CAG | GAG | CCT | GCT | CCA | AGT | TTT | CCT | CAG | TGA | GGC | CTT | TTG | CTG |
| 61  | C   | Q   | E   | P   | A   | P   | S   | F   | P   | Q   | *   |     |     |     |     |
| 226 | GAT | TGG | TCT | GAG | GAA | CAA | TTC | TGG | CTG | GAG | GTG | GGA | AGA | CGG | ATC |
| 271 | ACC | TCT | AAA | CTT | CTC | AAG | GAT | TTC | TTC | TAA | TAG | CTT | TGT | GCA | GAC |
| 316 | ATG | CGG | TGC | CAT | CAA | CAA | AAA | TGG | TCT | TCA | AGC | CTC | AAG | CTG | TGA |
| 361 | AGT | TCC | TTT | ACA | CTG | GGT | GTG | TAA | GAA | GGT | CAG | ACT | TTG |     |     |

Figure 3

|     |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
|-----|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 1   | ATG        | ACT        | GAC        | AGT        | GTT        | ATT        | TAT        | TCC        | ATG        | TTA        | GAG        | TTG        | CCT        | ACG        | GCA        |
| 1   | M          | T          | D          | S          | V          | I          | Y          | S          | M          | L          | E          | L          | P          | T          | A          |
| 46  | ACC        | CAA        | GCC        | CAG        | AAT        | GAC        | TAC        | GGA        | CCA        | CAG        | CAA        | AAA        | TCT        | TCC        | TCT        |
| 16  | T          | Q          | A          | Q          | N          | D          | Y          | G          | P          | Q          | Q          | K          | S          | S          | S          |
| 91  | TCC        | AGG        | CCT        | TCT        | TGT        | TCT        | TGC        | CTT        | GTG        | GCA        | ATA        | GCT        | TTG        | GGG        | CTT        |
| 31  | S          | R          | P          | S          | C          | S          | C          | L          | V          | A          | I          | A          | L          | G          | L          |
| 136 | CTG        | ACT        | GCA        | GTT        | CTT        | CTG        | AGT        | GTG        | CTG        | CTA        | TAC        | CAG        | TGG        | ATC        | CTG        |
| 46  | L          | T          | A          | V          | L          | L          | S          | V          | L          | L          | Y          | Q          | W          | I          | L          |
| 181 | TGC        | CAG        | GGG        | <u>ATT</u> | <u>TCT</u> | <u>TCT</u> | <u>AAT</u> | <u>AGC</u> | <u>TTT</u> | <u>GTG</u> | <u>CAG</u> | <u>ACA</u> | <u>TGC</u> | <u>GGT</u> | <u>GCC</u> |
| 61  | C          | Q          | G          | I          | S          | S          | N          | S          | F          | V          | Q          | T          | C          | G          | A          |
| 226 | <u>ATC</u> | <u>ACC</u> | <u>AAA</u> | <u>AAT</u> | <u>GGT</u> | <u>CTT</u> | <u>CAA</u> | <u>GCC</u> | <u>TCA</u> | <u>AGC</u> | <u>TGT</u> | <u>GAA</u> | <u>GTT</u> | <u>CCT</u> | <u>TTA</u> |
| 76  | I          | N          | K          | N          | G          | L          | Q          | A          | S          | S          | C          | E          | V          | P          | L          |
| 271 | <u>CAC</u> | <u>TGG</u> | <u>GTG</u> | <u>TGT</u> | <u>AAG</u> | <u>AAG</u> | <u>GTC</u> | <u>AGA</u> | <u>CTT</u> | <u>TGA</u> |            |            |            |            |            |
| 91  | H          | W          | V          | C          | K          | K          | V          | R          | L          | *          |            |            |            |            |            |

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Figure 4

|     |                                                             |
|-----|-------------------------------------------------------------|
| 1   | ATG GCC GAC AAC TCT ATC TAC TCA ACA TTA GAG CTG CCT GCT GCA |
| 1   | M A D N S I Y S T L E L P A A                               |
| 46  | CCT CGA GTC CAA GAT GAC TCC AGA TGG AAG GTC AAA GCT GTC TTA |
| 16  | P R V Q D D S R W K V K A V L                               |
| 91  | CAC CGA CCC TGT GTT TCC TAC CTT GTG ATG GTG GCT TTG GGG CTT |
| 31  | H R P C V S Y L V M V A L G L                               |
| 136 | TTG ACT GTG ATT CTC ATG AGT CTA CTG TTG TAC CAA CGG ACT CTG |
| 46  | L T V I L M S L L L Y Q R T L                               |
| 181 | TGC TGT GGC TCC AAG GGC TTT ATG TGT TCC CAG TGC TCC AGG TGC |
| 61  | C C G S K G F M C S Q C S R C                               |
| 226 | CCC AAC CTC TGG ATG AGG AAC GGG AGC CAC TGT TAC TAC TTC TCA |
| 76  | F N L W M R <u>N G S</u> H C Y Y F S                        |
| 271 | ATG GAG AAA AGG GAC TGG AAC TCT AGT CTG AAG TTC TGT GCA GAC |
| 91  | M E K R D W <u>N S S</u> L K F C A D                        |
| 316 | AAA GGC TCG CAT CTC CTT ACA TTT CCG GAC AAC CAG GGA GTG AAC |
| 106 | K G S H L L T F P D N Q G V N                               |
| 361 | CTG TTC CAG GAG TAT GTG GGC GAG GAC TTT TAC TGG ATT GGC TTG |
| 121 | L F Q E Y V G E D F Y W I G L                               |
| 406 | AGG GAC ATC GAT GGC TGG AGG TGG GAA GAT GGC CCA GCT CTC AGC |
| 136 | R D I D G W R W E D G P A L S                               |
| 451 | TTA AGC ATT CTC TCT AAC AGC GTG GTA CAG AAG TGT GGC ACC ATC |
| 151 | L S I L S N S V V Q K C G T I                               |
| 496 | CAC AGG TGT GGC CTC CAC GCC TCC AGT TGT GAG GTT GCT TTG CAG |
| 166 | H R C G L H A S S C E V A L Q                               |
| 541 | TGG ATC TGT GAG AAG GTC CTG CCC TGA                         |
| 181 | W I C E K V L P *                                           |